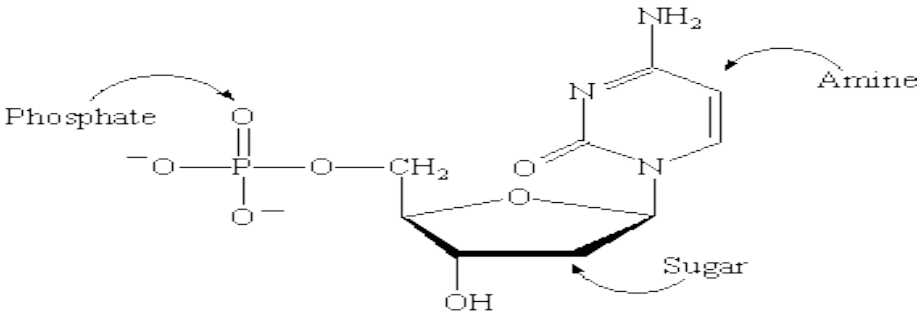
STUDIES ON METAL COMPLEXES AS NUCLEIC ACID BINDERS AND ENZYMATIC INHIBITORS

INTRODUCTION

Nucleic acids, which are relatively strong acids found in the nuclei of cells, were first isolated in 1869. The nucleic acids are polymers with molecular weights as high as 100,000,000 grams per mole. They can be broken down, or digested, to form monomers known as **nucleotides**. Each nucleotide contains three units: a sugar, an amine, and a phosphate, as shown in the figure below,

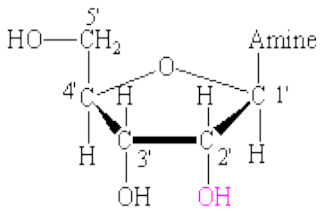


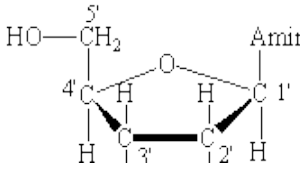
CLASSES OF NUCLEIC ACIDS

Nucleic acids are divided into two classes on the basis of the sugar used to form the nucleotides.

1. Ribonucleic acid (**RNA**) is built on a D-ribofuranose ring.

2. Deoxyribonucleic acid (**DNA**) contains a modified ribofuranose in which the -OH group on the second carbon atom has been removed, as shown in the fiugre below.

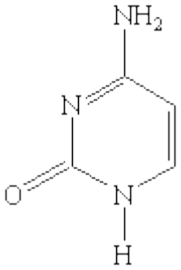




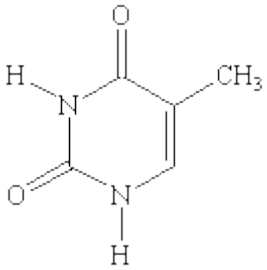
D-Ribofuranose found in DNA D-Deoxyribofuranose found in DNA

PURINES AND PYRIMIDINES

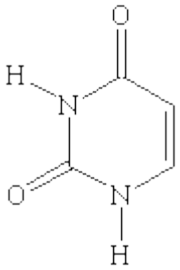
The amines that form nucleic acids fall into two categories: **purines** and **pyrimidines**. There are three pyrimidines cytosine, thymine, and uracil and two purines adenine and guanine, as shown in the figure below.



Cytosine

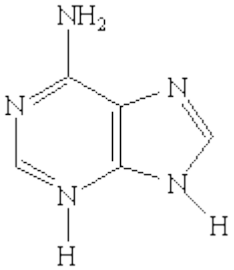


Thymine

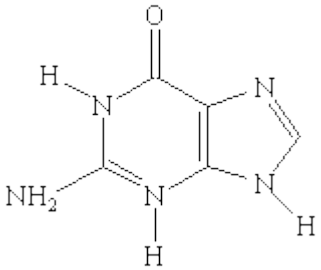


Uracil

PYRIMIDINES



Adenine



Guanine

PURINES

DNA and RNA each contain four nucleotides. Both contain the same purines adenine and guanine both also contain the pyrimidine cytosine. But the fourth nucleotide in DNA is thymine, whereas RNA uses uracil to complete its quartet of nucleotides.

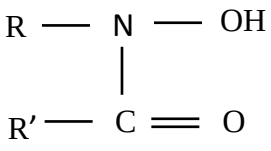
WHY NUCLEIC ACID IS STUDIED?

WORK on the structure and function of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) continues unabated in intensity. Due to their polyanionic nature, the natural nucleic acids DNA and RNA always occur in combination with cations. In living systems, these cations are usually polyamines as well as mono and divalent metal ions. It is probably the diversity of the possible interactions between nucleic acids and metal ions that has been fascinating scientists for a long time. The unwaning interest in this area of research became

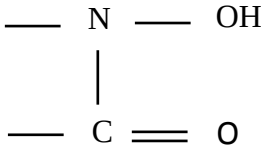
apparent in the recent publication of two monographs on nucleic acid interactions with metal ions and metal complexes (**1, 2**).

HYDROXAMIC ACIDS

Hydroxamic acids, the neutral polyfunctional molecules were first reported by H. Lossen (**3**) in 1869. In general, hydroxamic acids are the N-arylderivatives of hydroxylamine as illustrated in Fig.I, where R and R' are phenyl or differently substituted phenyl groups. NOH.C=O in Fig.II, the functional group is the pharmacophoric part of the molecule responsible for biological activity. These reagents show a wide spectrum of activities in analytical (**4, 5**), agriculture (**6,7**), technical (**8,9**) and medicinal fields (**10**).

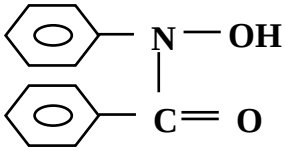


I



II

Hydroxamic acids show a wide spectrum of medicinal properties as reported from this and other laboratories (**11-21**). N-Phenylbenzohydroxamic acid (PBHA) first introduced by Bamberger in 1919 (**22**), is a parent compound of N-arylhydroxamic acid's series.



PBHA

Hydroxamic acids were mostly considered as classical chelating ligands and powerful enzyme inhibitors, progress in coordination chemistry during the two past decades has demonstrated that they can act as very efficient and versatile bridging ligands able to exhibit a variety of bridging coordination modes.(**23**). The richness of the coordination chemistry of hydroxamic acids stimulates extensive scientific researches in different areas of technology

PROPERTIES OF HYDROXAMIC ACIDS

- The outstanding property of hydroxamic acids is their ability to dissociate to a proton in slightly alkaline media (pKa~9). These allows for attachment of a metal ion.
- They undergo oxidation and reduction reaction with specific reagents e.g. Raney nickel, hydrogen gas and hydroiodic acid.
- Hydroxamic acids are weakly acidic compounds and do not liberate carbon dioxide from aqueous sodium bicarbonate solution.
- All the hydroxamic acids perform vanadium (V) test.

Hydroxamic acids (HA) are important bioligands, broadly utilized in medicine and chemical biology. Hydroxamic acid derivatives have been used as ligands in coordination chemistry of transition metals for a long time. The versatile biological activity of hydroxamic acids is due to their strong metal chelating ability and possibly of their NO-releasing properties (**24**).

ENZYMATIC ACTION

Enzyme is a protein molecule acting as catalyst in enzyme reaction. Enzyme inhibition is a science of enzyme-substrate reaction influenced by the presence of any organic chemical or inorganic metal or biosynthetic compound due to their covalent or non-covalent interactions with enzyme active site. Hydroxamic acids are well known to form strong complexes with a variety of transition metals, and their mechanism of inhibition involves binding to the metal ions of the active site of enzymes. Hydroxamic acids have therefore attracted much attention (**25**,**26**), and a large amount of hydroxamic acids were synthesized as inhibitors against metalloenzymes such as urease (**27**,**28**), matrix metalloproteinases (**29**), UDP-3-O-(R-3-hydroxymyristol)-Nacetyl-glucosamine deacetylase (**30**)and histone deacetylase(HDAC(**31**).

Molecular Modelling

Modelling is a tool for doing chemistry. Models are central for understanding of chemistry. Molecular modelling allows us to do and teach chemistry better by providing better tools for investigating, interpreting, explaining and discovering new phenomena.

A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD AND SIGNIFICANT CONTRIBUTION IN THE FIELD OF PROPOSED WORK

N-arylhydroxamic acids show biological activity as reported by this laboratory. Various QSAR parameters of these molecules have been determined and reported (**32,33**). The mode and the knowledge of DNA binding interactions with N-arylhydroxamic acids derivative is presently investigated but metal-chelating effect is unseen. Molecular Docking is also undertaken so that exact target point and mechanism of binding can be studied.

Therefore , present work will be focus on to know,

- How Hydroxamic acid metal complexes Interact with Nucleic Acids ?
- What effect can be observed with their interaction ?
- How Hydroxamic acid metal complexes inhibit enzyme activity ?

OBJECTIVE

In our laboratory, we have focused our attention to biomolecules /drug like molecules and their effect on target cellular DNA and RNA to understand the mechanism of action at the molecular level, and also to investigate the effect of thes molecules as anticancer and antitumor agent.

In the present investigation, the interaction of hydroxamic acids with nucleic acids will investigated following the techniques.

1. UV-Vis Spectroscopy
2. Fluorescence Spectroscopy
3. Gel Electrophoresis
4. Viscosity
5. Fluorescence Microscopic method

PROPOSED METHODOLOGY

The work will be distributed in the following chapters.

[1] INTRODUCTION

In this chapter, introduction of hydroxamic acids and nucleic acids will be described with recent literature survey data.

[2] SYNTHESIS OF HYDROXAMIC ACIDS

N-Arylhydroxamic acids and their derivatives will be synthesized in the laboratory following the steps reported in literature (**34**,**35**).

Following hydroxamic acids metal complexes will be applied for the present investigation

LIGAND- METAL COMPLEX

S. No.	HYDROXAMIC AICDS- METAL COMPLEXES
1.	Hydroxamic Acid – Aluminium Complex
2.	Hydroxamic Acid – Cobalt Complex
3.	Hydroxamic Acid – Copper Complex
4.	Hydroxamic Acid – Molybdenum Complex
5.	Hydroxamic Acid – Vanadium Complex

[3] DETERMINATION OF NUCLEIC ACID BINDING PARAMETERS

Following methods will be used to determine the Nucleic acid-binding parameters:-

1. BY UV-VIS ABSORPTION SPECTROSCOPY

The intrinsic binding constant of hydroxamic acids with DNA/RNA through UV absorption will be calculated from equation

$$[\text{Ct-DNA}]/(\varepsilon_a - \varepsilon_f) = [\text{Ct-DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad \textbf{[1]}$$

where,

Ct- DNA = base pair concentration

ϵ_b = apparent absorption coefficient for bounded DNA.

ϵ_f = apparent absorption coefficient for free DNA.

K_b = binding constant

2. VISCOSITY MEASUREMENT

DNA and RNA viscosity is sensitive to their length change, therefore its measurement upon addition of a compound is often concern as the least ambiguous method to clarify the interaction mode of a compound with both and provides reliable evidence for the intercalative binding mode .Viscosity measurements will be carried out with Ct-DNA and RNA solutions as a function of ligands concentration.This experiment is an effective tool to determine the binding mode of small molecules with DNA/RNA. The relative specific viscosity will be then obtained by Eq. **[2]**,

$$\eta / \eta_0 = (t-t_0)/(ctDNA-t_0) \quad \textbf{[2]}$$

where,

t_0 = flow time of buffer,

t_{DNA} = flow time of buffer containing DNA /RNA,

t = flow time of buffer containing DNA/RNA after the addition of hydroxamic acid,

η_0 = specific viscosity contributions of DNA/ RNA in the absence of complex,

η = specific viscosity contributions of DNA/RNA in the presence of the complex.

3. BY FLUORESCENCE QUENCHING METHOD

Fluorescence quenching constant will be obtained by using the Stern-Volmer equation **[3]**,

A quantitative estimation of the quenching in terms of the

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \qquad [3]$$

where,

F = fluorescence intensities of hydroxamic acid in absence of Hydroxamic acid

F = fluorescence intensities of hydroxamic acid in presence of the quencher,

K_{SV} = Stern-Volmer dynamic quenching constant,

[Q] = concentration of quencher.

4. COMPETITIVE BINDING WITH ETHIDIUM BROMIDE

Further support for the mode of binding between hydroxamic acids and DNA will be given through the competitive experiment. Here ethidium bromide (EB) can be employed in the examination of the reaction, as EB presumably binds initially to DNA by intercalation.The increase or decrease in the fluorescence intensity will provide the information regarding the nature of intercalation.

5. BY MELTING STUDIES

The double-helical structure of DNA is remarkably stable due to hydrogen bonding and base stacking interaction. Upon increasing the temperature the dissociation of helix to single strand increases. The melting temperature T_m, which defines as the temperature where half of total base pair is unbounded, will be determined as transition midpoint of melting curve (**29**) The change in T_m gives the information of interaction of hydroxamic acids to DNA/RNA.

6.THERMODYNAMIC PARAMETERS

The acting forces between small molecules and nucleic acid include, hydrogen bond, Van der Waals forces, hydrophobic interaction and electrostatic forces. According to Ross, binding forces are estimated by measuring ΔH and ΔS, following the Van't Hoff equation,

$$\textbf{Log K} = \Delta\textbf{H}/2.303\textbf{RT} + \Delta\textbf{S}/2.303\textbf{R} \qquad [4]$$

$$\Delta\textbf{G} = \Delta\textbf{H} - \textbf{T}\,\Delta\textbf{S} \qquad [5]$$

7. NUCLEIC ACID CLEAVAGE METHOD

Electrophoresis is a useful method for separating and detecting molecules such as protein and nucleic acid such as DNA and RNA. The nucleic acid cleavage ability of the compound will be investigated by gel electrophoresis technique. The results will be analyses by visualizing the gel with UV trans-illuminator (**36**, **37**).

8. FLUORESCENCE MICROSCOPY

Flourescence microscope is basically a light microscope that uses the fluorescence light eminating from fluorescent dye within the sample for imaging. Flourescence microscopy is an unique way of using a microscope to discover facts about specimen that often are not known by standard bright field microscopy. Here, specimen is self-illuminated by internal light, so bright objects are seen more easily. It can be used to find out the effect of the molecules on the higher-order structure of DNA/RNA through single-molecule observed in solution.

The diffusion constant, D for each nucleic acid molecule will be calculated (**38**-**39**) by using the following eq. **[6]**.

$$(\mathbf{R(t) - R(0)^2}) = \mathbf{4Dt + At^2} \qquad [6]$$

where,

$R(t) = (R_x, R_y)$ is the position of the centre of mass for a DNA and RNA,

$(R(t) - R(0)^2)$ = Mean square displacement,

A = Numerical constant related to convective flow.

The effective hydrodynamic radius of a single nucleic acid molecule will then be obtained from the Stokes-Einstein relationship

(**40**, **41**) given in eq. **[7].**

$$\mathbf{R_H = \frac{k_B T}{6\pi\eta_s D}} \qquad [7]$$

where,

k_B = Boltzmann constant,

η_s = Viscosity of the solvent.

DETERMINATION OF FREE RADICAL SCAVENGING ACTIVITY

The following four methods will be applied to measure the antioxidant property

(1) DPPH Method

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay method is based on the reduction of ethanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration, EC_{50} (**42**-**44**). The percent inhibition will be calculated from the following equation [8]:

$$\mathbf{\% \ Inhibition = [(A_0 - A_s / A_0) * 100]} \qquad \mathbf{[8]}$$

where,

A_0 = Absorbance of the blank solution,

A_s = Absorbance of the sample solution.

EC_{50} (Effective Concentration Value)

One parameter that has been introduced recently for the interpretation of the results from the DPPH method, is the "effective concentration" or EC_{50} value (also called the Inhibition Concentration, IC_{50} value).This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour). This parameter was apparently introduced by Brand-Williams and his colleagues (**45**, **46**).

(2) β -Carotene Linoleate Method

Oxidation of an aqueous emulsion of β-carotene and linoleic acid was employed as a test for measuring total antioxidant activity of the hydroxamic acids. In this particular model, heat induces formation of linoleic acid free radical. The presence of an antioxidant can reduce the extent of β-carotene destruction by reacting with the linoleate free radical or any other free radical formed within the system. Antioxidant activity will measured as the percentage of inhibition of lipid peroxidation in the β-carotene-linoleic acid system. According to the literature data (**47-52**), normalized antioxidant activity at 60 and 120 minutes of incubation (AA_{60} and AA_{120}) reflects the antioxidant activity more accurately.

The rate of β-carotene bleaching for sample will be calculated according to first-order kinetics [9]:

$$\mathbf{R = 1/T \times \ln A_0/A_t} \qquad \textbf{[9]}$$

where,

T = Time in minutes

A_0 = Initial absorbance

A_t = Absorbance after time interval, t (t = 15, 30, 45 minutes…etc.)

Based on the rates determined at three time intervals, the average rate of β-carotene degradation also be calculated. Furthermore, the antioxidant activity will be calculated from the absolute changes in absorbance at t = 60 and 120 min (AA_{60} and AA_{120}, respectively). Antioxidant activity will be measured as a percentage of inhibition of lipid peroxidation [**10**].

$$\mathbf{AA = \left[1 - \frac{R_t}{R_0}\right] x\ 100} \qquad \textbf{[10]}$$

where,

R_t = Rate of inhibition with sample,

R_0 = Control rate of inhibition.

(3) Ferrous Thiocyanate Method

The effect of compounds on the oxidation of linoelic acid using thiocynate method will be studied. The autoxidation of linoelic acid increases with increase in peroxide value. The difference in peroxide value can be found between the control (without antioxidant) and the linoelic acid containing compound will be studied to find out the antioxidant activity of compound. (**53,55**).

Molecular Modeling and Molecular Docking

These will be done by using following softwares like HEX , CoMFA , ARGUSLAB AUTOCAD etc some are explain below:

HEX

Hex is an interactive **protein docking** and **molecular superposition** program, written by Dave Ritchie. *Hex* understands protein and DNA/RNA structures in PDB format, and it can also read small-molecule SDF files. The parameters used for the docking process via Hex docking Software are,

- Correlation type – Shape and ESP
- FFT Mode – 3D
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

ARGUS LAB

Flexible docking will be done with ARGUS LAb .The ligand pdf structure will be drawn through chemical structure drawing software and the recetors (DNA , RNA) pdb file will be downloaded from Protein Data Bank(PDB). Then docking could be done through ASCORE method.

ENZYMATIC INHIBITORS

In recent years, there has been increasing interest in Hydroxamic acids as potent and selective inhibitors of a range of enzymes including matrix metalloproteases, (**56-60**)peroxidases, ,(**62-64)** hydrolases,(**65**,**66**) ureases,(**67-70**) lipoxygenases, (**56**)cyclooxygenasesand(**71**,**72**) peptide deformilases (**73**).

Enzymatic inhibitory activity can be determined by

- GEL ELECTROPHORESIS
- SPECTROPHOTOMETRIC METHOD
- FLUORESENCE METHODS
- HPLC
- CONDUCTOMETRIC METHOD
- DOCKING and many more techniques are available

GEL ELECTRPHORESIS

Electrophoretic separation of proteinases or proteinaceous proteinase inhibitors in the sample using sodium dodecyl sulfate-polyacrylamide gel slabs is followed by immersion of the gel in a protein substrate solution for detection of proteinases or an appropriate proteinase solution, and then in a protein substrate solution for detection of proteinase inhibitors.

SPECTROPHOTOMETRIC METHOD

The spectrophotometric method was developed after Holman and Burr Bergstrom

independently observed an increase in Ultra-violet light absorption ,when lipoxidase acted upon essential fatty acids**.**

FLUORESENCE SPECTROSCOPY

Fluorescence spectroscopy is generally used to calculate Enzyme-Complex fluorescence emission intensity with suitable fluorophore.

MOLECULAR DOCKING

Docking method is a powerful tool to explore ligand-receptor interaction providing 3D structure of the target. Currently docking software mainly includes

- Autodock,
- FlexX
- Gold .

EXPECTED OUTCOME OF THE PROPOSED WORK

Generally, organic molecules are less soluble in water but are more lipophilic and more permeable through membrane in their unionized form. It has been reported that biological activity of hydroxamic acids may be due to the similar structure present in proteins (**56**). Since, hydroxamic acids contain the smallest unit –C=O.NH, that can bind to DNA helix, therefore knowledge of favoured binding site and conformational aspects as well as its antioxidant properties are also important to understand the role played by hydroxamic acids in biological processes and cancer drug designing for anti-tumour / anti-cancer activity.

REFRENCES

1. .Hud, N.V.; *Royal Chemical Society Publishing* , 2009.
2. Hadjiliadis, N.; Sletten,E.; *John Wiley & sons*, 2009.
3. .Lossen, H.; *Analytical. Chem*istry, **150**, 314 (1869).
4. Tegonia, M.; Remelli, M.; *Coordination Chemistry Reviews*,**256**, 289 (2012).
5. Farina, Y.; A. Graisa A.; Yousif, E.; M. Kassim, M.; *Australian Journal of Basic and Applied Sciences*, **3** (1), 291 (2009).
6. Dimkpa, C.; Svatos, A.; Merten, D.; Buchel, G.; Kothe, E.; *Can. Journal of Microbioogy.*, **54**, 163 (2008).
7. Dubois, M.; Canadas, D.; Pernot, A.G.D.; Coste, C.; Leszkowicz, A.P.; *Journal Agricultural and Food Chem*istry, **56**, 1116 (2008).

8. Yang, Y. K.; Cho, H. J.; Lee, J.; Shin, I.; Tae.; *Journal of Oranometallic Letters.*, **11** (4), 859 (2009).

9. Zhu, B.Z.; Zhu, J.G.; Mao, L.; Katyanaraman, B.; Shan, G.Q.; *Proceedings of the National Academy of Sciences*, U.S.A., **15** (48), 13399 (2010).

10. Xia, J.; Chen, C.; Chen, Z.; Miele, L.; Sarkar, F.H.; Wang, Z.; *Biochimica et Biophysica Acta*, **1826**, 385 (2012).

11. Zuo, M.; Zheng, Y.W.; Lu, S.M.; Li, Y.; Zhang, S.Q.; *Bioorganic Medical Chemistry*, **20**, 4405 (2012).

12. Rho, H. S.; Baek, H. S.; Ahn, S. M.; Yoo, J. W.; Kim, D. H.; Kim , H. G.; Bull. *Korean Chemical Society*., **30** (2009).

13. Seo, S. K.; Jin, H. O; Lee, H. C; Woo, S. H.; E.-S. Kim, E.S.; Yoo, D. H. ; Lee, S. J.; Sungkwan, A.; Rhee, C.H.; Hong, S. I.; Choe, T. B.; Park., I. C.; *Molecular Pharmacology* , **73**, 1005 (2008).

14. Balliet, R. M.; Chen, G.; Gallagher, C. J. ; Dellinger, R. W. ; Sun, D.; Lazarus,P.; *Cancer Research*, **69**, 2981 (2009).

15. Kim, S. A.; Jin, Y. L. ; Kim, H. S.; Arch *Pharmacological Research*, **32** , 15 (2009).

16. Nguyen, A.; Su, L.; Campbell, B.; Poulin, N. M.; Nielsen, T. O.; *Sarcoma volume* (2009).

17. Butovich, I. A.; Lukyanova, S. M.; *Journal of Lipid Research*, **49**, 1284 (2008).

18. Lawrence, P.; Tardibono, Jr.; Miller, M. J.; *Organic Letters*, **11** , 1575 (2009).

19. Saban, N.; Bujak, M.; Cancer *Chemother Pharmacol*., **64**, 213 (2009).

20. Julija, M. S.; Ita, S. ; Lorena, H.; Blazenka, J. ; *Acta Pharm* ; **58** , 231 (2008).

21. Lossen, H.; *Analytical. Chem*istry, **150**, 314 (1869).

22. Bamberger, E.; Ber., **52**, 116 (1919).

23. .Golenya, I.A; Kontecka, E.G.; Haukka, M.; Oleksandr M.; Korsun. O.N.; Kalugin, N.; Fritsky, I.O.,*Cryst.Eng.Comm* ,**16** ,904,(2014)

24. Dzyuba, V.I.; Koval, L.I.; Bon,V.V.; Pekhnyo .V.I. ,*Polyhedron*, **29** , 2900–2906 (2010).

25. Zhang, Y.; Feng, J.; Jia, Y.; Wang, X.; Zhang,L.; Liu, C.; Fang, H.; Xu, W. *Journal of Medical Chemistry*, **54** (2011) .

26. Neelarapu, R.; Holzle, D.L.; Velaparthi, S.; Bai, H.; Brunsteiner, M.;S.Y. Blond, S.Y.; Petukhov, P.A., *Journal of Medical Chemistry*, **54** (2011) .

27. Muri, E.M.F.; Mishra, H.; Stein, S.M.; Williamson, J.S.; *Letter Drug Design Discovery* ,**1**, 30-34,(2004) .

28. Zaheer-Ul-Haq; Wadood, A.; Uddin,R.; *Journal of Enzyme Inhibition. Medicinal Chemistry*, **24,** 272-278, (2009).

29. , Perkovi,I.; Butula, I.; Zorc,B.; Hadjipavlou-Litina, D.,Pontiki,E.; Pepeljnjak, S.; Kosalec,I.; *Journal of Enzyme Inhibition. Medicinal Chemistry*, 24,1179-11879(2009).

30. Verma,R.P.; EXS, **103** 137-176(2012).

31. Brown,M.F.; Reilly,U.; Abramite, J.A.; Arcari,J.T.; Oliver,R.; Barham,R.A.; Che, J.M;Chen, E.M. ;Collantes, S.W.; Chung, C.; Desbonnet, J.; Doty, M.; Doroski, J.J. ;Engtrakul, T.M.; Harris, M. ;Huband, J.D.; Knafels, K.L.; Leach, S.; Liu, A.; Marfat,A. ;Marra, E. ;McElroy, M.; Melnick, C.A. ;Menard, J.I;. Montgomery, L.; Mullins, M.C.; Noe, J. O'Donnell, J.; Penzien, M.S.; Plummer, L.M. Price,V. ;Shanmugasundaram, C. ;Thoma, D.P.; Uccello, J.S.; Warmus, D.G., *Journal of Medicinal Chemistry*, **55** ,914-923(2012).

32. Guerrant, W.; Patil,V.; Canzoneri,J.C.; Oyelere, A.K.;*Journal of Medicinal Chemistry*, **55** (2012).

33. Yadav, S. S.;Pande,R.;Khare, D.; Tripathi, M.; *Journal of Chemical Thermodyn*amics **54**, 76 (2012).

34. Patre, S.; Thakur, P.; Pande, R.; *Journal of Chemical Thermodyn*amics., **54**, 118 (2012).

35. Gupta, V.K.; Tandon, S.G.; *Journal of Chemical Engineering.Data*, **17**, 248 (1972).

36. Pande, R.; Tondon, S.G.; *Journal of Chemical Engineering.Data* , **24**, 72 (1979).

37. Garcia-Gimenez,J.L.; Alzuet,G.; Gonzalez-Alvarez,M.; Liu-Gonzalez, M.;Castineiras, A.; Borras,J.; *J.ournalof Inoranic Biochem*istry **103**, 243–255 (2009).

38. Tong, C.; Hu, Z.; J. Fluore., **20**, 261 (2010).

39. Tarushi, A.; Psomas, G.; Raptopoulou,C.P.; Kessissoglou, D.P.; *Journal of Inorganic Biochem*istry,**103**, 898-905 (2009).

40. Chaveerach, U.;Meenogwa, A.;Trongpanich.; Soikum, C.; Chaveerach, P.;*Polyhedron* **29**, 731-738 (2010).

41. Bi, S. Y.; Zhang, H. Q.; Qiao, C. Y. ; Sun, Y.; Liu, C. M. ; *Spectrochimica Acta A*, **69**, 123-129 (2008).

42. Zhao, P.; Huang, J. W.; Mei, W. J.; He, J.; Ji, L. N.; *Spectrochimica Acta A*, **75**, 1108-1114 (2010).

43. Vani, T.; Rajani, M.; Sarkar, S.; Shishoo, C. J.; *International Research Journal Of Pharmacy*, **35**, 313-317 (1997).

44. Sanchez-Moreno, C.; Larrauri, J.; Saura-Calixto, F.; *Journal of Food Science Technology*., **79**, 1301-1304 (1999).

45. Navarro, M. C; **59**, 312-314 (1993).

46. Brand-Williams, W.; Cuvelier, M., E.; Berset, C.; *Food Science & Technology*., **28**, 25-30 (1995).

47. Bondet, V.; Brand-Williams, W.; Berset, C.; *Food Science & Technology*., **30**, 609-615 (1997).

48. Al-Saikhan, M. S.; Howard, L. R.; Miller Jr, J. C.; *Journal Food Science*., **60**, 341-343 (1995).

49. Amarowicz, R.; Pegg, R., B.; Rahimi-Moghaddam, P.; Barl, B., Weil, J. A.; *Food Chem*istry, **84**, 551-562 (2004).

50. Sarikurkcu, C.; Arisoy, K.; Tepe, B.; Cakir, A.; Abali, G.; *Food and Chemical Toxicology*, **47**, 2479 (2009).

51. Al-Saikhan, M. S.; Howard, L. R.; Miller Jr, J. C.; *Journal Food Sciences*., **60**, 341-343 (1995).

52. Koncic, M. Z.; Rajic, Z.; Petric, N.; Zorc, B.; *Acta Pharmceautica*, **59**, 235-242 (2009).

53. Nakatani, N.; Kikuzaki, H.; *Agricultural and Biological Chemistry*, **51**, 2727-2732 (1987).

54. Ali Ebrahimzadeh, M,; Nabavi, S. M.; Nabavi, S., F.; Bahramian, F.; Bekhradnia, A. R., Pak. *Journal of Pharmacological Sciences*, **23**, 29-34 (2012).

55. Rezaeizadeh, A.; Zuki, A.; Abdollahi, M.; Goh, Y. M.; Noordin, M. M.; Hamid, M.; Azmi, T.; *African Journal of Biotechnology*, **10**, 4932-4940 (2011).

56. Muri, E. M. ; Nieto, M. J. ; Sindelar, R. D. ; Williamson, J. S. ;Curr. Medicinal Chemistry, **9**, 1631-1653 ,(2002).

57. Steward, W. P. ; Thomas, A. L. ;Expert. Opin. Investig. Drugs,**9**, 2913-2922, (2000).

58. Botos, I. ; Scapozza, L. ; Zhang, D. ;. Liotta, L. A;. Meyer, E. F; Proc. Natl. Acad. Sci., USA, **93**, 2749-2754, (1996).

59. Sani, M. ; Belotti, D. ; Giavazzi, R. ; Panzeri, W. ; Volonterio, A.; Zanda, M. ,*Tetrahedron Letters* , **45**, 1611-1615,(2004).

60. Tegoni, M. ; Dallavalle, F. ; Santos, A. ,Journal of Inorgancic Biochemistry,**98**, 209-218,(2004).

61. Indiani, C. ; Santoni, E. ; Becucci, M. ; Boffi, A. ; Fukuyama, K.; Smulevich, G. ,*Biochemistry,* **42**, 14066-14074, *(*2003).

62. O-Brien, E. C. ; Farkas, E. ; Gil, M. J .; Fitzgerald, D. ; Castineras, A. ; Nolan, K. B. ,*Journal of Inorganic Biochemistry*,**79**,47-51,(2000).

63. Tsukamoto, K. ; Itakura, H. ; Sato, K. ; Fukuyama, K. ; Miura, S.;Takahashi, S. ; Ikezawa, H. ;Hosoya, T. ,*Biochemistry*, 38,12558-12568.(1999).

64. Brown, D. A. ; Glass, W. K. ; Fitzpatrick, N. J. ; Kemp, T. J.Errington, W.;Clarkson, G. J. ;Haase, W. ; Karsten, F. ;Mahdy, A. H.,*Inorganic Chimica Acta*, inpress.(2004).

65. Brown, D. A. ; Cuffe, L. P. ;Fitzpatrick, N. J. ; Ryan, A. T., *Inorganic Chemistry*, **43**, 297-302,(2004).

66. Amtul, Z. ;Rahman, A. U. ;Siddiqui, R. A. ;Choudhary, M. I.; *Curriculum Medicinal Chemistry*, **9**, 1323-1348,(2002).

67. Benini, S. ; Rypniewski, W. R. ; Wilson, K. S. ; Miletti, S. ;Ciurli, S.;Mangani, S. ,*Journal of BioInorganic Chemistry*, **5**, 110-118,(2005).

68. Brown, D. A. ; Cuffe, L. P. ; Fitzpatrick, N. J. ; Glass, W. K.; Herlihy, K.; Namir, H. ; Deeg, O. ; Errington, W. ; Kemp, T. J. ;*Chemical Commununication*, 2433(1998).

69. Arnold, M. ; Brown, D. A. ; Deeg, O. ;Errington, W. ; Haase, W.; Herlihy, K.; Kemp, T. J. ;Nimir, H. ;Werner, R. ,*Inorganic Chemistry* **37**, 2920,(1998).

70. C. M. Dooley, M. Devocelle, B. McLoughlin, K. B. Nolan, D. J. Fitzgerald, C. T. Sharkey, *Molecular Pharmacology*, **63**, 450-455.(2003)

71. Connolly, P. J. ;. Wetter, S. K ; Beers, K. N. ; Hamel, S. C. ;Chen, R. H.; Wachter, M. P. ; Ansell, J. ; Singer, M. M. ; Steber, M. ;Ritchie, D. M. ;. Argentieri, D. C, *Bioorganic Medicinal Chemistry Letters*,**9**, 979-984,(1999).

72. Chen, D. ; Hackbarth, C. ; Ni, Z. J. ; Wu, C. ; Wang, W. ; Jain, R. ; He, Y.; Bracken, K. ; Weidmann, B. ;. Patel, D. V .; Trias, J;. White, R. J.;Yuan, Z. ; *Antimicrobial Agents Chemotherapy*, **48**, 250-261,(2004).

73. Leao, A.; Hoekman, D.; *Perspective in Drug Discovery and Design*, **18**, 19 (2000).

R. Pande
30.4.14

Manta

Signature of the Supervisor

Dr. (Mrs.) RAMA PANDE
PROFESSOR
SCHOOL OF STUDIES IN CHEMISTRY
PT. RAVISHANKAR SHUKLA UNIVERSITY
RAIPUR-492010, CHHATTISGARH, INDIA

Signature of the Candidate

Forwarded

30.4.2014

Chairman, DRC

CHAIRMAN
D. R. C.
School of Studies in Chemistry
Pt. Ravi Shankar Shukla University
RAIPUR (C.G.)